

A common feature of hematologic malignancies, particularly myelodysplastic syndrome (MDS), is the presence of mutations in spliceosome genes, which occur in up to 50% of patients with MDS. The spliceosome is a key regulator of pre-mRNA processing, and as such spliceosome mutations are correlated with dysregulated RNA processing and an increased prevalence of alternatively-spliced RNA isoforms. Spliceosome (SF) mutants cause alternative splicing isoforms that could produce novel protein isoforms with neomorphic protein functions. We previously found misplicing of the DNA damage response gene, ATR, occurred in MDS-associated U2AF1-mutant cells and in patients with unknown significance. The CRISPR-Cas13d system has been shown to be capable of targeting mRNA using an engineered guide RNA sequence. Preliminary work suggests the ability to target isoform-specific RNA with the Cas13d system, however there is minimal evidence of the best approach to designing isoform-specific guides and the efficacy of this system in knocking down alternatively-spliced isoforms. We are utilizing guide-RNA designing algorithms and molecular cloning techniques to target different exonic sequences and exon-exon junctions in RNA transcripts to determine the efficacy of Cas13d in isoform-specific knockdown and to establish a system to target mis-spliced RNA variants. This will provide a new tool to characterize the function of mis-spliced genes in MDS and other cancers harboring spliceosome gene mutations.